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Polymorphism of waxy proteins in Spanish hulled wheats

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Abstract

Hulled wheats are neglected crops that have potential in plant breeding programmes of modern durum and common wheat. Among these wheats, three species were widely cultivated in Spain until the mid 20th century: *Triticum monococcum* ssp. *monococcum* (einkorn), *Triticum turgidum* ssp. *dicoccum* (emmer) and *Triticum aestivum* ssp. *spelta* (spelt). One important aspect of wheat grain quality is starch composition, which is related to the action of waxy proteins. A collection of 536 accessions of Spanish hulled wheats was analyzed for waxy protein composition using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Polymorphism was found for the Wx-A1, Wx-B1 and Wx-D1 proteins, including new and null alleles in the three species. An allelic variant with an electrophoretic mobility not previously described was found in einkorn wheat. In emmer and spelt, some alleles with different mobility were also found. A *Wx-B1* null allele was detected in emmer wheat, and null alleles for *Wx-A1*, *Wx-B1* and *Wx-D1* were found in spelt wheat. The variations found could be used to enlarge the gene pool available to breeders, and to design new cultivars with different levels of amylose content.

Keywords: amylose content; genetic polymorphism; hulled wheats; waxy proteins

Introduction

Hulled wheats are the wild or cultivated species of the *Triticum* genus, which have glumes that tightly enclose the grains, even after normal threshing. In Spain, three species of hulled wheats were widely cultivated until the late 1960 s: *Triticum monococcum* L. ssp. *monococcum* ($2n = 2x = 14$, AA), *T. turgidum* ssp. *dicoccum* Schrank em. Thell. ($2n = 4x = 28$, AABB) and *T. aestivum* ssp. *spelta* L. em. Thell. ($2n = 6x = 42$, AABBDD). Nowadays, only emmer and spelt wheat are still cultivated, and solely in marginal farming areas of Asturias (North of Spain). Fortunately, some of the biodiversity that once existed is conserved in germplasm banks. Due to the growing interest in natural food, some of these ancient crops are undergoing a revival in European agriculture. In the last decade, our research group has investigated three important collections of Spanish hulled wheats, mainly for genetic variation in seed storage proteins (Caballero *et al.*, 2001, 2004a, b; Pflüger *et al.*, 2001; Alvarez *et al.*, 2006). The high variability detected in these species suggests that these collections are useful gene reservoirs that can be used in breeding programmes.

The starch composition of wheat grain has a primary influence on flour quality. Wheat starch consists of two types of glucose polymers, the essentially linear amylose and the highly branched amylopectin, in a ratio of 22–35:68–75%. Synthesis of amylose in the seed endosperm is carried out by the waxy proteins or granule-bound starch synthases that are encoded by genes

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located in the *Wx* locus on the homoeologous group 7 chromosomes (Ainsworth *et al.*, 1993). In common wheat, the *Wx-A1* locus is located on chromosome 7AS, the *Wx-B1* locus on 4AL (a segment of chromosome 7BS that has been translocated) and the *Wx-D1* locus on chromosome 7DS. Since starch properties such as gelatinization, pasting and gelation depend on the amylose: amylopectin ratio (Zeng *et al.*, 1997), these proteins are very important in terms of flour quality.

The aim of this study was to assess waxy protein polymorphism in a broad collection of Spanish hulled wheats.

Material and methods

Plant material

In the current study, 536 Spanish hulled wheat lines (29 lines of einkorn, 87 lines of emmer and 420 lines of spelt) were analyzed. These lines were derived by single seed selection from an equal number of accessions obtained from the National Small Grain Collection (Aberdeen, MD, USA), the Center for Genetic Resources (The Netherlands) and the Centro de Recursos Fitogenéticos-INIA (Alcalá de Henares, Spain). The plants were grown under field conditions during 2008, and several spikes per plant were protected to prevent random crossings. Durum wheat cultivars (cvs). Langdon and Mexicali and common wheat cultivar (cv). Chinese Spring were used as standards.

Starch extraction and electrophoretic analysis

Twenty milligrams of flour were mixed with 1 ml of distilled water and incubated at 4°C for 24 h. The homogenate was filtered through Miracloth and centrifuged at 14,000 g for 1.5 min. The pellet was washed with 1 ml of buffer A (55 mM Tris–HCl, pH 6.8, 2.3% (w/v) SDS, 2% (w/v) dithiothreitol (DTT) and 10% (v/v) glycerol), according to the method of Echt and Schwartz (1981). Then, 1 ml of buffer A was added to the pellet and left for 30 min at room temperature. The pellet was washed three times with distilled water, once with acetone and then air-dried. The residue was mixed with 80 µl of buffer A, heated in a boiling water bath for 2 min, cooled on ice and centrifuged.

Aliquots of supernatant (20 µl) were loaded in vertical SDS-PAGE slabs in a discontinuous Tris–HCl–SDS buffer system (pH: 6.8/8.8) at a polyacrylamide concentration of 12% (w/v, cross-linker (C): 0.44%). The Tris–HCl/glycine buffer system of Laemmli (1970) was used. Electrophoresis was performed at a constant current of 30 mA/gel at 18°C, continuing for 4 h after the tracking dye migrated off the gel. Protein bands were visualized by silver staining.

For two-dimensional PAGE (2D-PAGE), 8.0 mg of starch was soaked at room temperature in 300 µl of lysis buffer (8 M urea, 2% ampholine pH 3.5–10 (Pharmacia) and 5% DTT). After centrifugation, the supernatant containing the solubilized proteins was subjected to 2D-PAGE using isoelectric focusing (IEF) for the first dimension and SDS-PAGE for the second. IEF gels contained 2.5% (v/v) ampholines (pH 3–10/5–8 and 8–10, 1:1). Focusing commenced from the acidic end (0.01 M H_3PO_4) at 200 V for 30 min, and continued at 400 V for 17 h, and then at 800 V for 1 h at room temperature. After IEF, SDS-PAGE was conducted as described above.

Results and discussion

The studies carried out on waxy proteins polymorphism have shown that variability is relatively low compared with other cereal grain proteins, such as seed storage proteins. However, Yamamori *et al.* (1994, 1995) found five alleles for the *Wx-A1* gene in common wheat, and another six alleles have been described for the *Wx-B1* gene in common and durum wheats (Rodríguez-Quijano *et al.*, 1998). More recently, Caballero *et al.* (2008) reported high variability for these proteins in some ancient wheats and related species.

Ten waxy alleles (two for *Wx-A^{m}1* locus, two for *Wx-A1*, three for *Wx-B1* and three for *Wx-D1*) were detected in the lines herein evaluated (Table 1). In all cases, one of these alleles was clearly predominant, while the others were rare or very rare. For the *Wx-A^{m}1* locus, the allele *Wx-A^{m}1a′* that exhibited lower mobility than the *Wx-A^{m}1a* was only found in a single accession (BGE-014269). Both alleles showed higher mobility than that of the *Wx-A1a* allele detected in polyploid wheat

Table 1. Variability and allelic frequencies (in brackets) detected for waxy genes in the three collections evaluated

Locus	Alleles	Einkorn ($n = 29$)	Emmer ($n = 87$)	Spelt ($n = 420$)
Wx-A^{m}1	a	28 (96.6)	–	–
	a′	1 (3.4)	–	–
Wx-A1	a	–	87 (100)	384 (91.4)
	b	–	0	36 (8.6)
Wx-B1	a	–	85 (97.8)	317 (75.5)
	b	–	1 (1.1)	49 (11.7)
	c′	–	1 (1.1)	54 (12.8)
Wx-D1	a	–	–	418 (99.6)
	b	–	–	1 (0.2)
	g	–	–	1 (0.2)
H_e		0.067	0.023	0.189

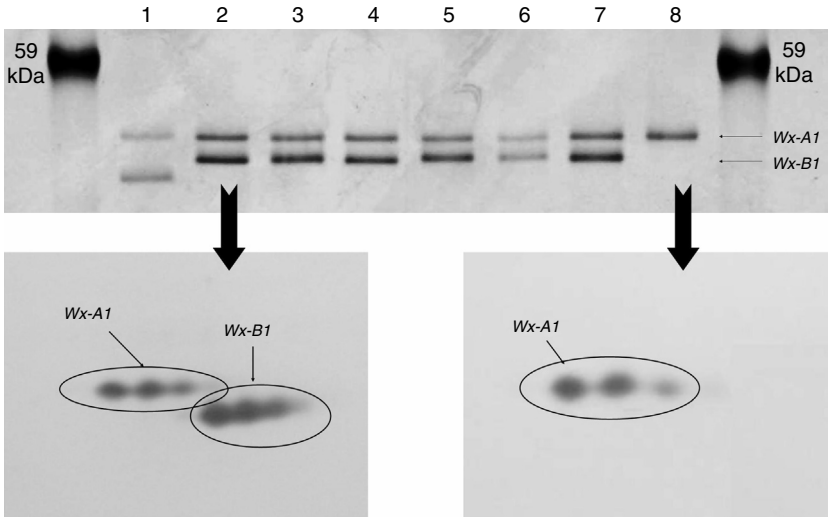

Fig. 1. Electrophoresis separation of waxy proteins in tetraploid wheats. One-dimensional SDS-PAGE (up) and two-dimensional IEF × SDS-PAGE (down). Lanes are as follows: 1, KU 4213D (*Wx-A1a* and *Wx-B1d*); 2 and 4, Emmer-71 (*Wx-A1a* and *Wx-B1a*); 3 and 7, cv. Langdon (*Wx-A1a* and *Wx-B1a*); 5, cv. Mexicali (*Wx-A1a* and *Wx-B1c*^{l}); 6, Emmer-49 (*Wx-A1a* and *Wx-B1c*^{l}); 8, Emmer-52 (*Wx-A1a* and *Wx-B1b*).

(emmer and spelt). This is in agreement with studies that suggest that the A genome of einkorn is substantially different from the A genome present in emmer and spelt (Dvorak *et al.*, 1993).

Emmer did not show polymorphism for the *Wx-A1* locus, whereas in spelt 36, accessions that presented the *Wx-A1b* allele (null type) were detected. The *Wx-A1a* alleles detected in both species were similar to the allele detected in the durum and common wheat cultivars used as standards (Langdon and Mexicali, and Chinese Spring, respectively).

The variability for the *Wx-B1* locus was higher in both polyploid species. However, most of the emmer accessions present the *Wx-B1a* allele, similar to the alleles found in cvs. Langdon and Chinese Spring. The other two alleles were very rare. Because SDS-PAGE can occasionally generate overlapping bands that yield misleading results, the accessions that presented the null allele were analyzed by two-dimensional electrophoresis (IEF × SDS-PAGE). The data revealed that the *Wx-B1* gene is not expressed in this accession (Fig. 1).

The results were very similar for the *Wx-D1* locus, with all accessions showing the *Wx-D1a* allele with exception of one accession that presented the *Wx-D1b* allele and another a novel allele named *Wx-D1g,* which exhibited a slightly lower electrophoretic mobility than the *Wx-D1a* allele.

The mean expected heterocigosity (H_e) values were very low for all species (Table 1), representing approximately 13.4, 5.5 and 48.6% of the genetic diversity for einkorn, emmer and spelt, respectively, if the allelic variants of each locus were distributed randomly.

In conclusion, the current study has shown that Spanish hulled wheats exhibit significant waxy protein variation, with some novel alleles at risk of erosion by genetic drift. Consequently, the safeguarding of these and other hulled wheat accessions stored in germplasm banks is fundamental for the maintenance of genetic diversity. This diversity may be of use in breeding programmes focussed on starch quality, for both modern wheats and these ancient crops that are undergoing such a revival.

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